Modelling and Simulation of Citric Acid Production from Corn Starch Hydrolysate Using Aspergillus Niger

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Abstract

The kinetics of citric acid fermentation from corn starch hydrolysate using *Aspergillus niger* ATCC 9142 was studied in a batch fermenter. A general model for citric acid production was formulated. Four kinetic models, Monod, Haldane, logistic and hyperbolic for describing the growth of the fermenting microorganism were explored. The validity of the models in terms of predicting growth of the fermenting organism was determined by fitting each kinetic model to experimental data collected in the course of this work. Comparison of experimental results to model predicted results showed that only the hyperbolic model was able to accurately replicate the experimental results. This was evident from the high level of correlation between the experimental and model predicted results. The kinetic parameters for cell growth, substrate consumption and product formation μ_{max}, $Y_{x/s}$, $Y_{p/x}$, K_s and K_p as calculated by the hyperbolic model are 0.01320h^{-1}, 0.711g/g, 13.6708g/g, 0.0006g/dm^3, and 0.2572 g/dm^3 respectively. The validated model was implemented in an advanced equation oriented modelling software to determine the effect of key process parameters on the production of citric acid. Results of simulating the model show that the production of citric acid is a growth associated process. Optimum pH, initial sugar concentration and temperature of for citric acid production were 5.5, 40*w/v* and 30^oC respectively.

Keywords: Citric acid, Fermentation, Corn starch, Hydrolysate, Modelling, *Aspergillus niger*

1. Introduction

Citric acid is present in essentially all plants and in many animal tissues and fluids. It is a constituent of wine, milk, cheeses and it is abundant in most citrus fruits such as oranges, tangerines, lemon, berries, lime etc. It is also a metabolic product formed in the citric acid or Krebs cycle (Nadeem, Syed, Baig, Irfan & Nadeem, 2010).

Citric acid has an estimated annual production rate of about 1.4 to 1.5 million tons per year and its demand rate is estimated to be growing at a rate of about 3.5 to 4.0% annually (Lancini, 2008; Pandey, Soccol, Rodriguez-Leon & Nigam, 2001; Soccol, Luciana, Berghe, Cristine & Pandey, 2006). Of the total amount of citric acid produced annually, about 70% is utilised by the food industry because of its pleasant acid taste, high solubility in water, chelating, antioxidant and buffering properties. About 12% is utilised by pharmaceutical industries as liquid elixirs, flavouring, anticoagulant and preservative while the remaining 18% is utilised by other industries such as cosmetics, toiletry, detergent, textile, oil recovery, paper etc (Archer, 2000; Hang & Woodams, 1985; Pandey *et al*., 2001; Sarangbin, Kirimura & Usami, 1993; Soccol, Prado, Vandenberghe & Pandey, 2003).

It has been demonstrated that producing citric acid from synthetic or chemical methods cannot compete favourably with biotechnological means (Papagianni, 2007; Max, Salgado, Rodríguez, Cortés, Converti &

Domínguez, 2010; Nadeem *et al*., 2010). Hence, a large proportion of the world's demand for citric acid is satisfied from biotechnological sources.

Wehmer (1893) first demonstrated that the filamentous fungi *Penicillium* can produce citric acid in a culture medium containing sugars and inorganic salts. With the passage of time, many other microorganisms that can produce citric acid have been discovered. These include strains of *Aspergillus niger, Aspergillus awamori, Aspergillus nidulans, Mucor piriformis, Penicillium janthinellum Trichoderma viride* etc. Though these microorganisms can produce citric acid, *Aspergillus niger* has been used for the production of citric acid since the second decade of the twentieth century and has remained the most used organism for commercial production of citric acid (Nadeem *et al*., 2010; Papagianni, 2007). Production of citric acid from *Aspergillus niger* has many advantages over the use of other strains in the sense that *Aspergillus niger* is easy to handle, can ferment a variety of cheap feedstock producing yields in the excess of 70% of theoretical values.

The source of carbon substrate for citric acid fermentation has been the object of a lot of studies, especially with respect to the use of polysaccharides such as starch. It is generally accepted that only sugars that are quickly metabolised by the fermenting microorganism results in a high final yield of citric acid (Mattey, 1992). This makes it preferable to use feedstock containing simple sugars. In this category is sugarcane either in the form of cane juice or cane molasses. This form is the most important feedstock utilized in tropical and sub-tropical countries for producing citric acid. In European countries, beet molasses are the most utilized sucrose-containing feedstock (Cardona & Sánchez, 2007). Because of the simple form in which these sugars exist, it is easy to convert them to citric acid via fermentation. The major challenge faced by this source of feedstock for citric acid production is the competition created by the use of this same feedstock as food. There are indications that continued use of this source can affect food prices in the long run.

Unlike simple sugars, bioconversion of starch to citric acid is not a direct process. This is because it is a complex polysaccharide. It is necessary to hydrolyse the starch in order to produce fermentable sugars which can then be converted to citric acid by a suitable microorganism. Starch hydrolysates are obtained from starch by acid and enzyme hydrolysis. Hydrolysates are filtered to remove suspended solids and insoluble impurities.

In trying to study and understand the dynamic behaviour of a process, it is important to formulate dynamic models of such processes. These models upon calibration and validation will provide insights as to how the process functions, how it responds to changes in operating procedure and how amenable it is to control. Mathematical models of most processes encountered in chemical engineering are nonlinear in nature (Suja Malar & Thyagarajan, 2009).

The usefulness of a dynamic mathematical model in analysing complex processes cannot be over emphasized. Dynamic modelling and simulation of processes leads to vast improvements in process economics, design, operation and control (Lee, Wang & Newell, 1997). Model predictions also make it possible to identify optimal design and operational parameters and this consequently leads to the maximisation of the system's performance (Andres, Hu, Snowling & Schraa, 2006).

In this work, the concept of dynamic behaviour of process systems and how a formulated model can be used to predict the behaviour of a process is presented. The case considered for investigation was the production of citric acid from acid and enzyme hydrolysed corn starch using *Aspergillus niger*. Experimental data from batch fermentation of citric acid was analysed to in order to develop a model that can replicate the experimental data. The objectives of the work are:

- To develop a general model for citric acid production from hydrolysed corn starch using *Aspergillus niger.*
- Explore kinetic models for describing microbial growth.
- To validate the model by estimating kinetic parameter and comparing model predictions with experimental data.
- To explore by simulation, the effect of key operating variables on the performance of the process.

The validated model will be implemented in an advanced equation oriented process modelling software gPROMS. Simulation of the validated model will provide insight as to how the process responds to changes in operating procedure and how certain operating variables affect the process.

2. Materials and Methods

2.1 Microorganism

Aspergillus niger ATCC 9142 was obtained from the biotechnology division of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. It was maintained on Potato Dextrose Agar (PDA) slants and stored in a refrigerator at 4^{o}C until it was needed.

2.2 Substrate and pretreatment

Industrial grade corn starch was obtained from the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. 40% w/v corn starch was prepared by mixing 30g of corn starch with 100cm^3 of 0.1M hydrochloric acid. The mixture was autoclaved at 121^{o}C for a period of 15 minutes. The mixture was removed from the autoclave and allowed to cool to ambient temperature and 1.0 M sodium hydroxide was added to stop the acid hydrolysis reaction. The hydrolyzed starch was filtered and the filtrate was collected for citric acid production.

2.3 Culture medium, inoculum and fermentation

The constitution (g/dm^3) of the fermentation medium used for citric acid production was as described by Lotfy, Ghanem, & El-Helow, (2007). Glucose, 40.0; $NaNO_3$, 4.0; KH_2PO_3, 1.0; $MgSO_4.7H_2O$, 0.23; $FeCl_3$, 0.02; $ZnSO_4$, 0.0012; $MnCl_2.H_2O$, 0.0012. The pH of the culture medium was adjusted to 5.5 by adding a sterile solution of hydrochloric acid.

Conidia suspensions of fungal strains were obtained from cultures grown on potato dextrose agar slants at 30 ^{o}C for 5 to 7 days. The spores were washed with sterilized 0.8% Tween 80 solution by shaking vigorously for 1 minute. Spores were counted with a haemocytometer to obtain approximately 10^8 spores/ cm^3.

Surface fermentation was carried out in 250 cm^3 Erlenmeyer flasks. The flask containing the fermentation medium was inoculated with 0.5 cm^3 of the inoculum and then incubated at 30^{o}C.

2.4 Analyses

Liquid samples were taken at intervals of 24 hours and analysed for glucose, biomass, citric acid and pH. Cell concentration was measured by dispensing 5 cm^3 of fermentation broth into a tube and centrifuging it at 5000 rpm for 30 minutes. The optical density of the sample was measured spectrophotometrically at 600nm and compared to standard curve of dry weight of *Aspergillus niger* cells. The glucose content of the sample was determined using the method of Miller (1959). The citric acid content of the sample was determined using the method of Marier & Boulet (1958). The pH of the sample was determined using a Unican 9450model pH meter.

3. Batch Fermenter Model Formulation

In order to predict the citric acid productivities of *Aspergillus niger* in a batch fermenter, a mathematical model was developed. The parameters for cell growth, substrate consumption and citric acid formation kinetics were estimated as part of the model validation exercise. The model was used to estimate cell, substrate and citric acid concentrations respectively as well as dynamic response of the batch fermentation process. The development of the model involved deriving expressions for material, energy balances and microbial growth rates.

3.1 Material balance

In carrying out the material balance, a batch fermenter is considered. This is approximated as a perfectly mixed continuous stirred tank reactor (CSTR) of constant volume.

3.1.1 Substrate balance

In carry out an overall balance for the substrate, the following assumptions were made.

- The fermenter is of constant volume
- The fermenter is perfectly mixed

Overall substrate balance about the fermenter is given as:

$$\frac{dS}{dt} = -q_s X \tag{1}$$

S (g/dm^3) is the concentration of substrate in the fermenter while X (g/dm^3) is the concentration of biomass in the fermenter. The specific substrate consumption rate q_s (*g substrate/g biomass/h*) is given by a modified form of the maintenance model proposed by Pirt, (1965).

$$q_s = \frac{\mu}{Y_{x/s}} \tag{2}$$

Where $Y_{x/s}$ (*g biomass/g substrate*) is the biomass yield.

3.1.2 Biomass balance

In carrying out an overall balance for the biomass, it is assumed that microbial growth is only limited by the availability of organic substrate rather than oxygen and the endogenous decay of cells is negligible.

The overall biomass balance about the fermenter is given as:

$$\frac{dX}{dt} = \mu X \tag{3}$$

Where μ (h^{-1}) is the specific growth rate. For the specific growth rate, kinetic models will be explored to determine the one that best fit the experimental data. These models are defined as follows.

Monod model: (Monod, 1949)

$$\mu = \mu_{\max} \frac{S}{K_s + S} \tag{4}$$

Haldane model: (Andrews, 1968)

$$\mu = \mu_{\max} \frac{S}{K_s + S + (S^2 / K_I)} \tag{5}$$

Hyperbolic model: (Novak, Strehaiano, Moreno & Goma, 1981)

$$\mu = \mu_{\max} \frac{S}{K_s + S} \frac{K_p}{K_p + P} \tag{6}$$

Logistic equation: (Baei, Mahmoudi & Yunesi, 2010)

$$\frac{dX}{dt} = \mu_{\max} X \left(1 - \frac{X}{X_{\max}}\right) \tag{7}$$

Where μ_{max} (h^{-1}) is the maximum specific growth rate of biomass, K_s (g/dm^3) is the substrate affinity constant, K_p (g/dm^3) is the product inhibition constant, K_i (g/dm^3) is the substrate inhibition constant and X_{max} (g/dm^3) is the maximum concentration of biomass.

3.1.3 Product balance

For a batch operation, product balance about the fermenter is given as:

$$\frac{dP}{dt} = q_p X \tag{8}$$

In equation 8, $P\,(g/dm^3)$ is the concentration of citric acid while the specific rate of citric acid production q_p (*g citric acid/g biomass/h*) is given by the Luedeking-Piret – like model presented in equation 9.

$$q_p = Y_{p/x} \mu \tag{9}$$

$Y_{p/x}$ (*g citric acid/g biomass*) is the citric acid yield.

3.2 Energy balance

The components of the general energy balance equation applied to a fermenter include the following:

- Energy generated by metabolism, Q_{met}
- Heat loss via by aeration, $Q_{aeration}$
- Energy required for agitation, Q_{agit}
- Heat loss via evaporation, Q_{evap}
- Heat loss via convection, Q_{conv}
- Heat duty associated with feed inlet, Q_{feed}

The general energy balance equation as applied to a fermenter is expressed mathematically as:

$$\rho V C_p \frac{dT_b}{dt} = Q_{met} + Q_{aeration} + Q_{agit} + Q_{evap} + Q_{conv} + Q_{feed} \tag{10}$$

For a batch system without agitation and neglecting convective losses, the general energy balance equation reduces to:

$$\rho_l V_l C_{pl} \frac{dT_b}{dt} = Q_{aeration} + Q_{met} - Q_{evap} \tag{11}$$

The term on the left hand side is the energy accumulated in the system. All terms on the right hand side are defined as follows.

$$Q_{aeration} = G\rho_g C_{pg}(T_b - T_g) \tag{12}$$

$$Q_{met} = V_l \Delta H_{met} \tag{13}$$

$$Q_{evap} = G\lambda_w(H_{go} - H_{gi}) \tag{14}$$

Combining (12) to (14) with (11) results in:

$$\rho_l V C_{pl} \frac{dT_b}{dt} = G\rho_g C_{pg}(T_b - T_g) + V_l \Delta H_{met} - G\lambda_w(H_{go} - H_{gi}) \tag{15}$$

The metabolic heat ΔH_{met} is defined as:

$$\Delta H_{met} = \frac{\mu X}{Y_{x/s}}(\Delta H_{c.s} - Y_{x/s}\Delta H_{c.x}) \tag{16}$$

In equation 14, λ_w (J/m^3) and G (m^3/s) are respectively the specific latent heat of vaporization of water and flow rate of inert aeration gas into the fermenter while H_{go} and H_{gi} represent the specific humidities of the outlet and inlet gas streams respectively and they are defined as follows:

$$H_{go} = \frac{18}{28}\frac{P_{vo}}{P_{atm} - P_{vo}} \tag{17}$$

$$H_{gi} = \frac{18}{28}\frac{P_{vi}}{P_{atm} - P_{vi}} \tag{18}$$

The vapour pressures of the outlet and inlet gas streams (P_{vo} and P_{vi}) are given by their respective Antoine equations.

$$P_{vo} = exp\left[18.3 - \frac{3816.4}{T_{go} - 46.1}\right] \tag{19}$$

$$P_{vi} = exp\left[18.3 - \frac{3816.4}{T_{gi} - 46.1}\right] \tag{20}$$

T_{go} and T_{gi} are the temperatures of the outlet and inlet gas streams.

4. Results and Discussion

4.1 Model validation and parameter estimation

The batch fermenter model was validated against experimental data collected in the course of this work. This was done by estimating parameters of the different kinetic models under consideration. Table 1 shows the parameters estimated and their respective optimal estimate for the respective kinetic models considered. Estimation of the maximum specific growth rate (μ_{max}) by the hyperbolic model and logistic equation resulted in fairly similar values. The Monod and Haldane models resulted in dissimilar values. For the substrate affinity constant (K_s), all the models gave very different results. Fairly similar values were obtained for the product and biomass yield for all models. These parameters were used to generate time profiles of substrate, biomass and product concentrations for each model.

Figures 1 to 4 show the overlay plots which displays the comparison between experimental data and the model predicted data for substrate (sugar), biomass (dry *A. niger* cells) and product (citric acid) concentrations for Monod, Haldane, logistic and hyperbolic models respectively. It can be observed from Figure 1 that the Monod model was able to replicate the values of substrate and product concentrations fairly well but it performed poorly in predicting the concentration of biomass. Results obtained for the Haldane and logistic models as shown in Figures 2 and 3 show that both models were able to predict the concentration of substrate fairly well but performed poorly in predicting the concentration of product and biomass. Also, Figure 4 shows the high level of correlation between the experimental results and model predicted results obtained for the hyperbolic model. The model was able to replicate the concentrations of substrate, biomass and product as obtained from experiment. This is an indication that the model exhibits a good fit with the experimental data. The hyperbolic model is therefore able to correctly model the kinetics of substrate consumption, cell growth and product formation during citric acid production from hydrolysed corn starch.

4.2 Substrate consumption

For sugar consumption by *Aspergillus niger,* Figure 4 shows that there was a gradual reduction in the residual sugar content from an initial value of 3.032 g/dm^3 at the start of fermentation to 0.152 g/dm^3 at the end of fermentation, which is after 120hours. The reduction observed is as a result of the consumption of the sugar by the fermenting organism. Our findings are in agreement with those reported by Baei *et al*., (2008). According to them, they observed similar decrease in the residual sugar content as a result of citric acid formation by the metabolic consumption of sugar obtained from apple pomace by *A. niger*. The parameters for sugar consumption $Y_{x/s}$ and K_s as obtained from the hyperbolic model were 0.0711g/g and 0.0006 g/dm^3 respectively.

4.3 Microbial growth

Figure 4 illustrates the growth of the fermenting organism, *A. niger*. It was observed that there is an increase in the concentration of *A. niger* cells from 0.10 g/dm^3 at the start of fermentation to a maximum of 0.36 g/dm^3 at about 96hours. Between 96hours and 120hours, there is a reduction in the concentration of fermenting organism to 0.26 g/dm^3. The trend observed indicates that there is growth of the fermenting organism between 0 and 96 hours of fermentation while there is a decline in growth between 96 and 120 hours. The decline observed is usually as a result of the substrate being used up and also probably due to the accumulation/presence of toxic substances in the fermentation vessel that might inhibit the action of the fermenting organism. The parameters for *A. niger* growth μ_{max}, $Y_{x/s}$ and K_s as calculated by the hyperbolic model were 0.01320h^{-1}, 0.0711g/g and 0.0006 g/dm^3 respectively.

4.4 Product formation

Figure 4 shows the time profile of citric acid concentration. It was observed that the bio-production of citric acid is almost linear with respect to cell growth from the start of fermentation till about 96hours. This is an indication that citric acid formation is growth associated. The concentration of citric acid produced increased steadily from zero to about 2.7 g/dm^3 after which it levels off at 96hours. These observations were also in agreement those of Nadeem *et al*., (2010). In their investigation into the enhancement of citric acid production, they recorded similar reduction in citric acid production after attaining a maximum value. The decrease in citric acid productivity observed is due to the decline in the growth of fermenting organism at 96 hours since the formation of citric acid is growth associated. It might also be as a result of inhibitory effect of high citric acid concentration, reduction in the nitrogen in the fermentation medium and depletion of sugar source as reported by Al-Sheri & Mostafa, 2006, Alvarez-Vasquez, Gonzalez & Torres, 2000, Arzumanov, Shishkanova & Finogenova, 2000 and Kristiansen & Sinclair, 1978. The parameters for citric acid production $Y_{p/x}$ and K_p *as* calculated by the hyperbolic model were 13.6708g/g and 0.2572 g/dm^3 respectively.

4.5 Effect of process parameters on citric acid production

The validated hyperbolic model was used to simulate the effect of process parameters on citric acid production. The parameters used for simulation were those calculated by the hyperbolic model.

4.5.1 Effect of pH

The effect of pH on the production of citric acid is illustrated in Figure 5. It was observed that the concentration of citric acid increased with pH up to a maximum at a pH of 5.5 after which it declined. The results show that the best citric acid concentration of 1.94 g/dm^3 was obtained at a pH of 5.5. The pH is important in two respects. Firstly, spore germination which is required for fermentation requires a pH of 5 and above to occur. Secondly, protons are released when ammonia is absorbed by germinating spores. This causes a release of hydrogen ions thus lowering the pH of the medium. The low pH has the effect of improving citric acid production and providing a close to sterile environment which reduces the risk of contamination (Max *et al*., 2010).

4.5.2 Effect of initial sugar concentration

The effect of initial sugar concentration on the production of citric acid is shown in Figure 6. The initial sugar concentration is a very important parameter in citric acid fermentation. For the batch fermentation process under consideration in this work, it is observed that the citric acid concentration increases with initial sugar concentration up to a maximum of 2.326 g/dm^3 at an initial sugar concentration of 40*w/v* after which it declines and levels off.

4.5.3 Effect of temperature

The effect of temperature on the production of citric acid is illustrated in Figure 7. The trend observed is such that there is an increase in the production of citric acid between 25^{o}C and 30^{o}C after which production decreases. The best citric acid concentration of 1.431 g/dm^3 was obtained at a temperature of 30^{o}C. This was the optimum

temperature identified for citric acid production. The fermentation temperature is important in that when cells are grown under non ideal temperature conditions, they exhibit signs of adverse growth and metabolic production (Ellaiah, Srinivasulu & Adinarayana, 2004). Nampoothiri, Baiju, Sandhya, Sabu, Szakacs & Pandey, (2004) reported that citric acid production could be affected by a slow germination of the fungi, slow metabolic activity, enzyme denaturation and reduced cell viability when *Aspergillus niger* cells are incubated under low or high temperatures. The optimum temperature obtained in this study is in agreement with the fact that filamentous fungi such as *Aspergillus niger* are mesophilic thus requiring optimal temperatures between 25°C and 35°C for growth (Reid, 1998; Suresh & Chandrasekaran, 1999).

5. Conclusions

In this work, the concept of the dynamic behavior of a process and how a formulated dynamic model can be used to investigate and predict the behavior of such a process has been introduced. The case investigated was the batch fermentation of citric acid from corn starch hydrolysate using *A. niger*.

The following conclusions can be drawn from this study:

- A validated hyperbolic model which incorporates a product inhibition term was able to predict how the concentration of substrate, biomass and product in the batch fermenter varies with time to a relatively high level of confidence. This is evident in the high level of correlation between the experimental results and the model predicted results.
- The production of citric acid is growth associated. This is evident from the observation that citric acid formation is almost linear with respect to cell growth from the start of fermentation till about 96hours.
- The performance of the different kinetic models; that is the ability to replicate the experimental results is ranked as follows: hyperbolic model > Monod model > Haldane model > logistic equation.
- The optimum pH, initial sugar concentration and temperature for citric acid fermentation were 5.5, 40w/v and 30^{o}C respectively. These were the conditions at which the best citric acid production.

A modelling exercise such as this is necessary because the resulting model serves as a readily available tool for analysing fermentation processes. The effect of changes in operating conditions can easily be investigated without resorting to carrying out further experiments. This ultimately leads to considerable cost savings.

Nomenclature

C_{pl}	Liquid phase specific heat capacity (*J/Kg.* ^{o}C)
C_{pg}	Aeration gas specific heat capacity (*J/Kg.* ^{o}C)
G	Aeration gas flow rate (l/h)
H_{gi}	Specific humidities of the inlet gas stream (-)
H_{go}	Specific humidities of the outlet gas stream (-)
K_I	substrate inhibition constant (g/l)
K_P	product inhibition constant (g/l)
K_s	Half saturation constant (g/l)
P	Product (citric acid) concentration (g/l)
P_{vi}	Vapour pressures of the inlet gas stream (Pa)
P_{vo}	Vapour pressures of the outlet gas stream (Pa)
P_t	Atmospheric pressure (Pa)
Q_{met}	Heat generated by metabolism (W)
$Q_{aeration}$	Heat loss via by aeration (W)
Q_{agit}	Heat duty of agitation (W)
Q_{evap}	Heat loss via evaporation (W)
Q_{conv}	Heat loss via convection (W)
Q_{feed}	Heat duty associated with feed inlet (W)
q_s	Specific substrate consumption rate (*g substrate/ g cells. h*)

q_p Specific production rate (*g product/ g cell. h*)

S Substrate (sugar) concentration (g/l)

T_b Temperature of broth (^oC)

T_{gi} Temperature of the inlet gas streams (^oC)

T_{go} Temperature of the outlet gas stream (^oC)

V_l Volume of liquid in the fermenter (l)

X Biomass (cell) concentration (g/l)

X_{max} Maximum concentration of biomass (g/l)

$Y_{p/x}$ Product yield (*g product/ g cells*)

$Y_{x/s}$ Biomass yield (*g cells/ g substrate*)

Greek letters

ρ_l Liquid phase density (g/l)

ρ_g Density of aeration gas (g/l)

ΔH_{met} metabolic heat (kJ/g)

$\Delta H_{c,s}$ Heat of combustion of substrate (kJ/g)

$\Delta H_{c,x}$ Heat of combustion of biomass (kJ/g)

μ Specific growth rate (1/h)

μ_{max} Maximum Specific growth rate (1/h)

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Table 1. Values of estimated parameters using different models

Parameter	Optimal Estimate			
	Monod model	Haldane model	Hyperbolic model	Logistic equation
$\mu_{\max}(h^{-1})$	0.0227	0.0527	0.0132	0.0183
$K_s(g/dm^3)$	1.622	5.0000	0.0006	N/A
$Y_{p/x}$(g/g)	14.099	12.8550	13.6708	14.3000
$Y_{x/s}$(g/g)	0.0743	0.0773	0.0711	0.0763
$K_I(g/dm^3)$	N/A	7.6069	N/A	N/A
$K_P(g/dm^3)$	N/A	N/A	0.2572	N/A
$P_m(g/dm^3)$	N/A	N/A	N/A	3.00
$X_m(g/dm^3)$	N/A	N/A	N/A	0.445

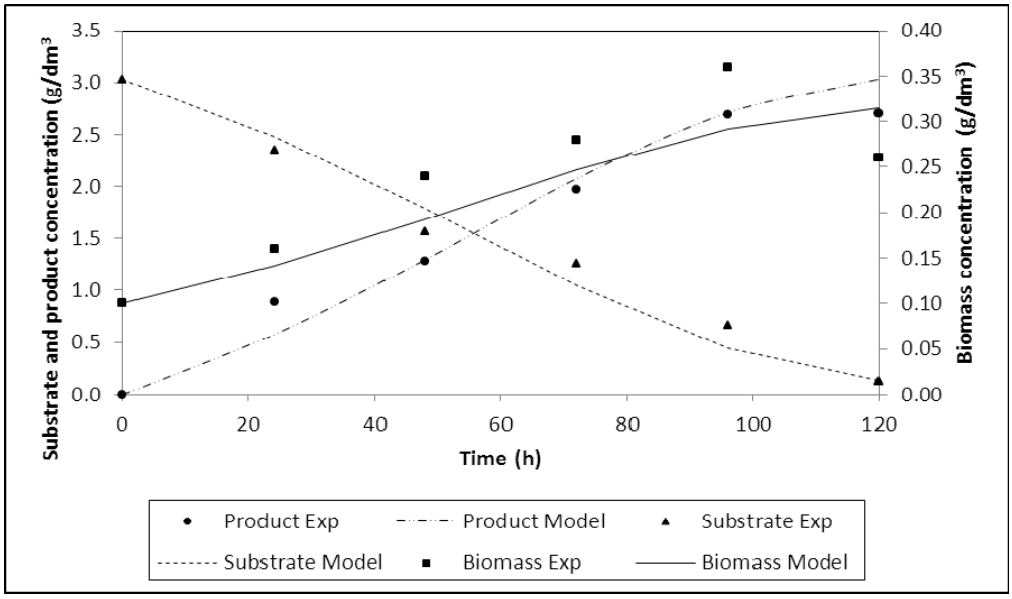

Figure 1. Comparison between experimental data and the model predicted data for substrate, biomass and product concentrations for Monod model

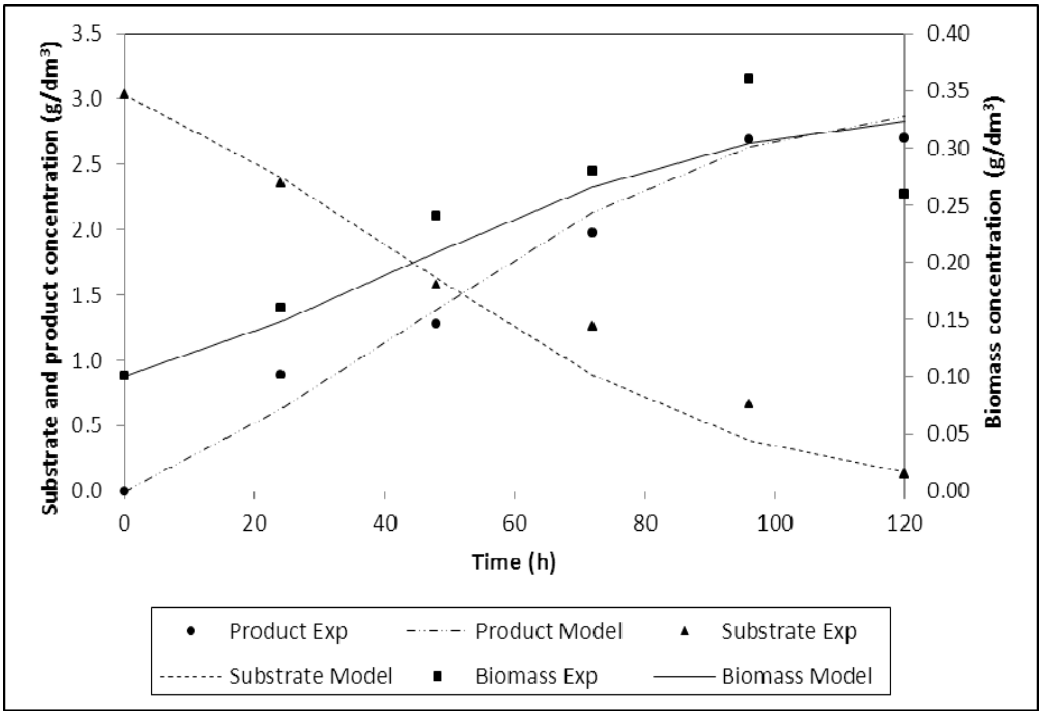

Figure 2. Comparison between experimental data and the model predicted data for substrate, biomass and product concentrations for Haldane model

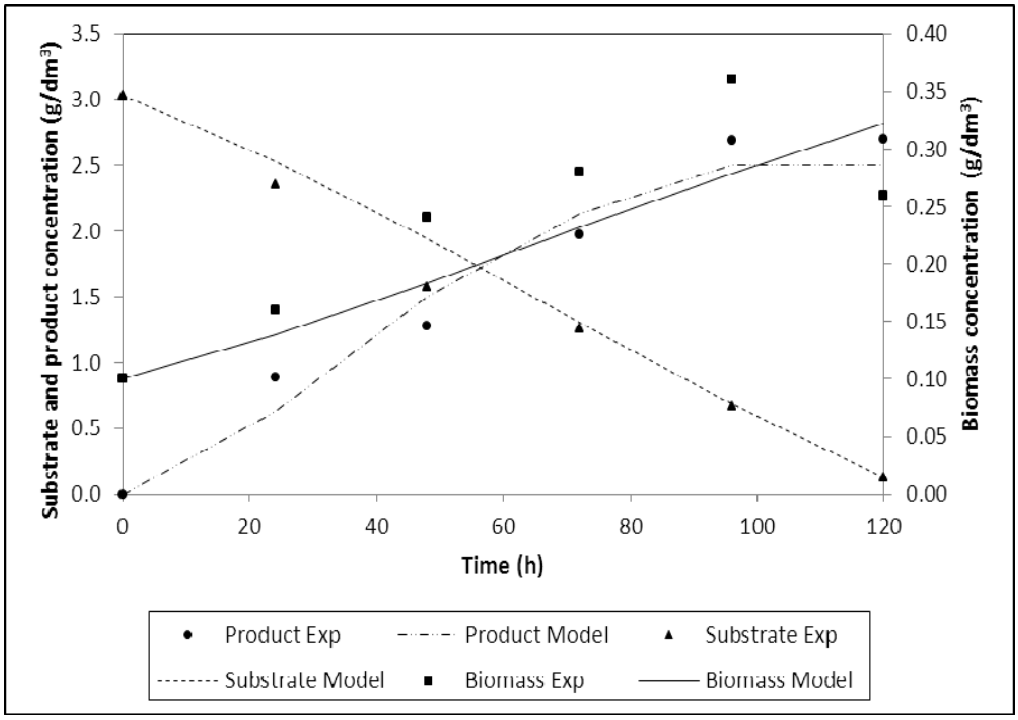

Figure 3. Comparison between experimental data and the model predicted data for substrate, biomass and product concentrations for logistic equation

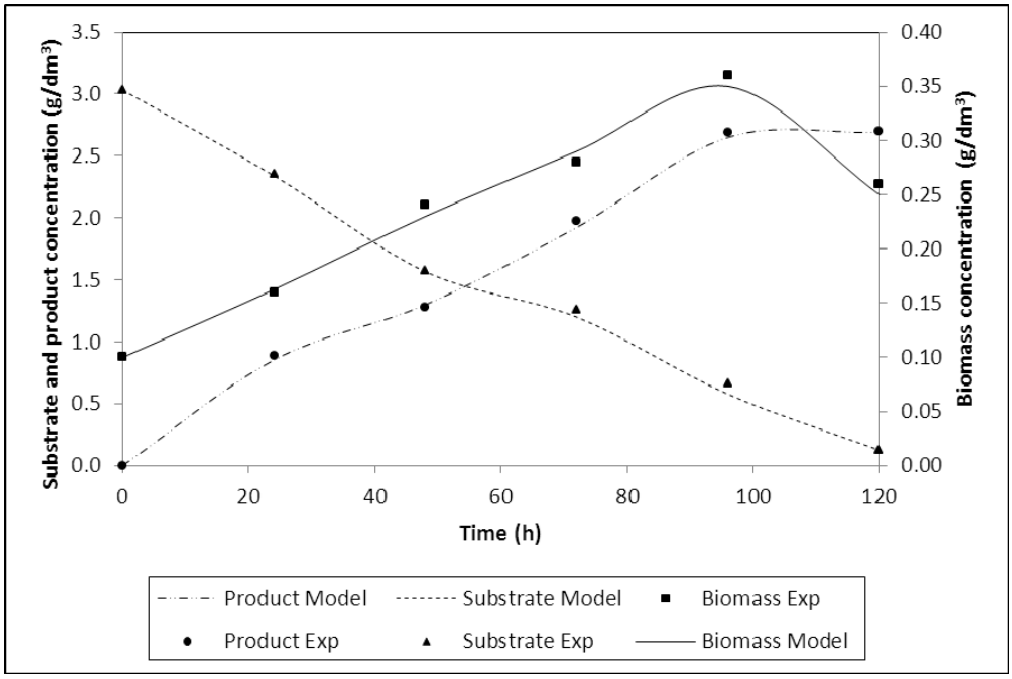

Figure 4. Comparison between experimental data and the model predicted data for substrate, biomass and product concentrations for hyperbolic model

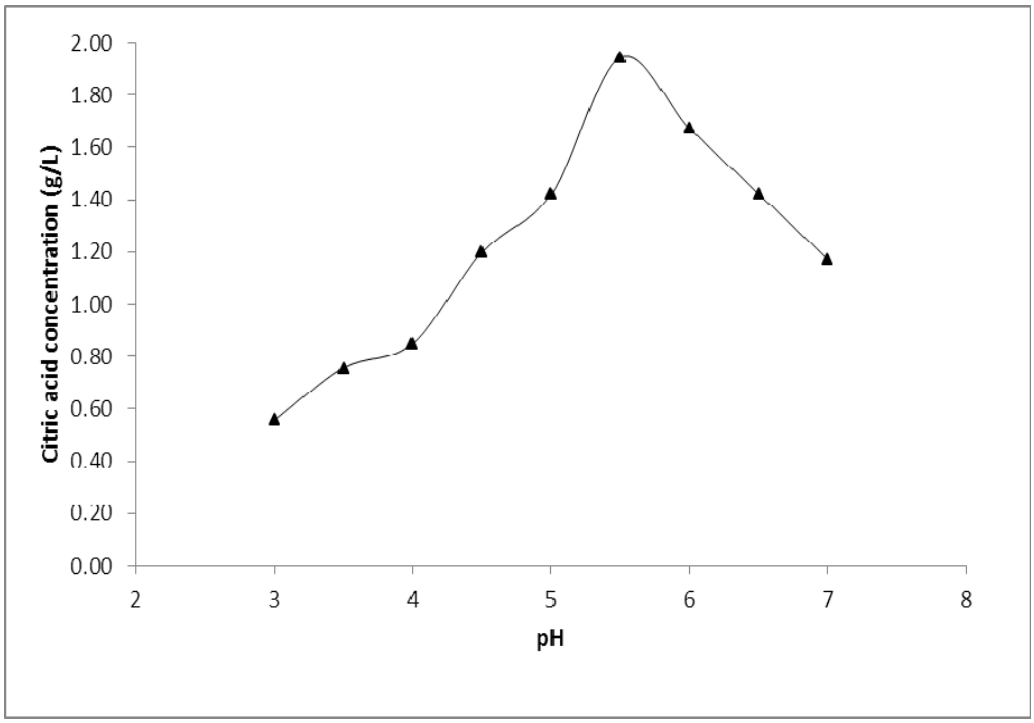

Figure 5. Variation of citric acid concentration with pH

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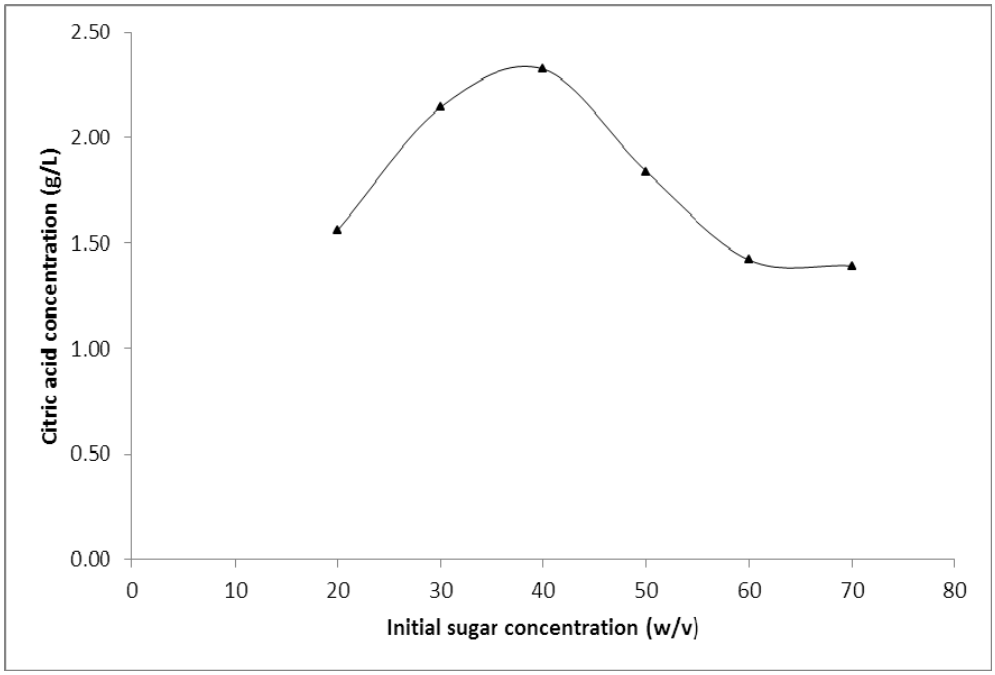

Figure 6. Variation of citric acid concentration with initial sugar concentration

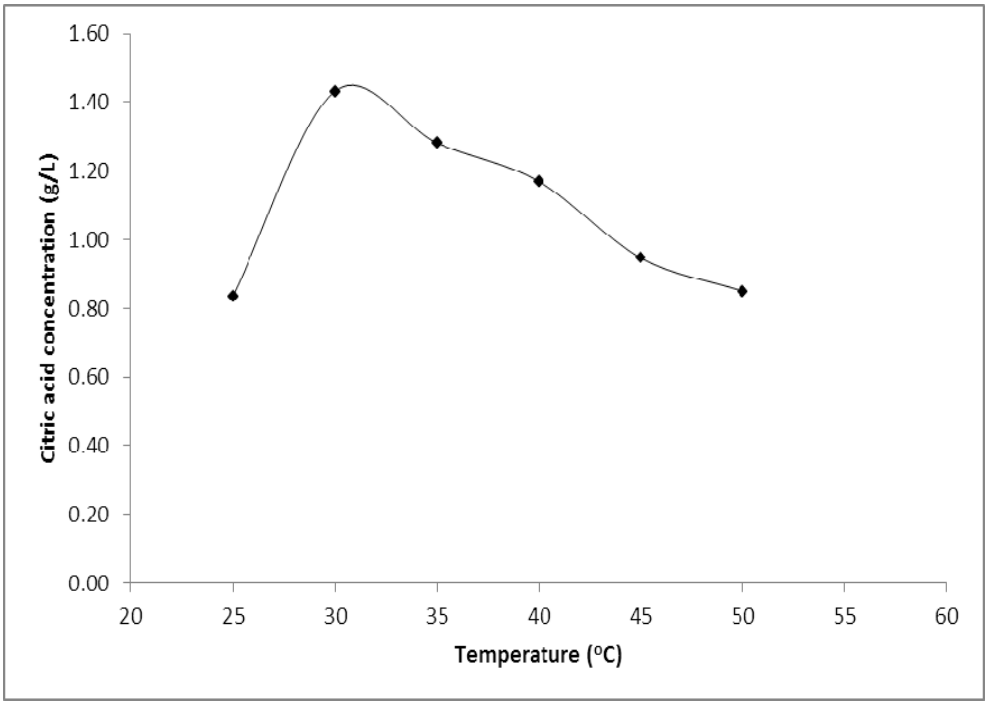

Figure 7. Variation of citric acid concentration with temperature